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TranSignal™ PDZ Domain Arrays

Cat. # MA3020, MA3022, MA3023, & MA3024
Product User Manual
Released 09/25/03

Panomics *Transcending boundaries...*

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1. ARRAY OVERVIEW

Panomics' TranSignal™ PDZ Domain Arrays include a total of 123 different human PDZ domains. These arrays are made using the recombinant conserved binding sites of individual PDZ domain proteins fused with glutathione-S-transferase (GST). Proteins are affinity-purified and immobilized onto a membrane. Each PDZ domain on the arrays is spotted in duplicate.

For a complete map and list of the domains included on the arrays, see Appendices A, B, C, and D.

2. INTRODUCTION & BACKGROUND

PDZ Domain Structure & Function

A key to understanding cellular signal transduction is clarifying how proteins interact with one another. Protein-protein interactions are often mediated by noncatalytic, conserved domains. One of these domains is the PDZ domain.

PDZ domains are regions of sequence homology found in diverse signaling proteins (1–3). The name "PDZ" derives from the first three proteins in which these domains were identified: PSD-95, a protein involved in signaling at the post-synaptic density; DLG, the *Drosophila* Discs Large protein; and ZO-1, the zonula occludens 1 protein (1–3). PDZ domains are also sometimes called DH domains or GLGF repeats.

PDZ domains act as modules for protein-protein interactions. PDZ domains recognize specific motifs that occur at the C-termini of target proteins; these motifs are most often present in the cytoplasmic tails of transmembrane receptors and channels (4, 5). PDZ domains can also recognize internal sequences (6–9).

By recruiting downstream proteins in a signaling pathway, PDZ domains mediate assembly of specific multi-protein complexes. Proteins that contain PDZ domains play important roles in many key signaling pathways, including maintaining epithelial cell polarity and morphology (10, 11), organizing the postsynaptic density in neuronal cells, and regulating the activity and trafficking of membrane proteins (12).

Valuable Tool for Studying PDZ Domains

Now that the draft sequence of the human genome is complete, we are faced with myriad new proteins whose functions remain a mystery. An important step toward characterizing the function of a PDZ domain-interacting ligand and/or protein is to identify to which PDZ domain it binds, and hence determine with which signaling pathway it is involved. Traditional methods for detecting protein-protein interactions, such as co-immunoprecipitation, are arduous and time consuming at best. Panomics offers a convenient tool for identifying interactions between a ligand and PDZ domains that remain folded in active conformations.

With Panomics' TranSignal PDZ Domain Arrays, you can determine whether your protein of interest binds to multiple PDZ domains—all in one experiment. The assay couldn't be simpler: just express your protein of interest in bacteria and incubate the extract with the TranSignal PDZ Domain Array membrane. The protein interactions literally take place on the array membrane, and you can visualize them using HRP-based chemiluminescence detection.

The TranSignal PDZ Domain Arrays can also be used when the ligand of interest is available as a biotinylated synthetic peptide (see Appendix D) or purified protein when the appropriate secondary antibody for detection is available. Biotin uses anti-streptavidin HRP conjugate and purified protein uses an antibody specific to the protein followed by a corresponding HRP-conjugate.

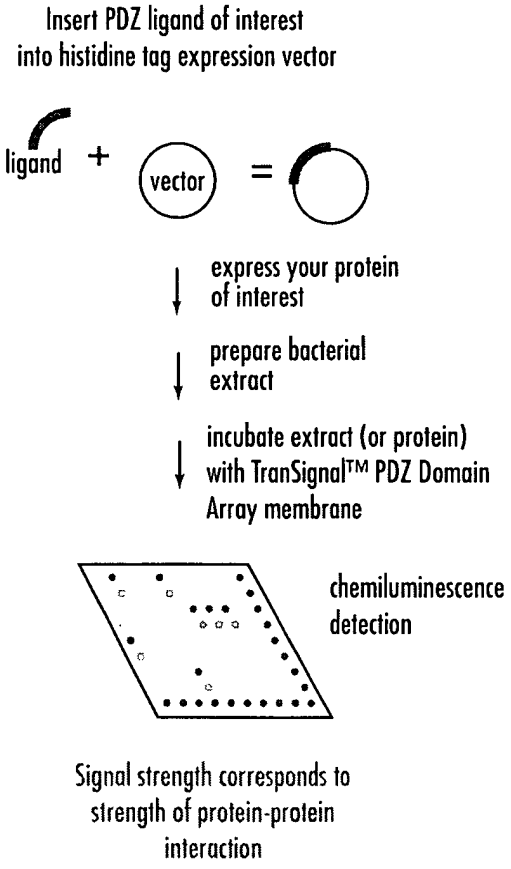


Figure 1: Flow chart of the TranSignal™ PDZ Domain Array assay.

3. MATERIALS PROVIDED

STORAGE CONDITONS: Upon receipt, the array membranes; Anti-Histidine HRP Conjugate; Blocking and Wash Buffers; and Detection Buffers A & B should be kept at 4°C until use. The PDZ Control Bacterial Extract should be stored at –20°C.

- **TranSignal PDZ Domain Array** (2 each; 3 cm wide x 6 cm long)
- **20X PDZ Control Bacterial Extract** (500 µl)
  (From *E. coli* expressing PDZ ligand)
- **400X Anti-Histidine HRP Conjugate** (20 µl)
- **1X Blocking Buffer** (15 ml)
- **1X Resuspension Buffer** (60 ml)
- **10X Wash Buffer** (15 ml)–dilute to 1X with dH_20
- **Detection Buffer A** (600 µl)
- **Detection Buffer B** (600 µl)
- **Four-Well Tray**

Sufficient quantities of each buffer are provided for two assays.

4. ADDITIONAL MATERIALS REQUIRED

3.1 Reagents and Solutions

- **Histidine Tag Expression Vector** (pET Systems from Novagen *or* pQE Systems from Qiagen)
- **DH5α Competent Cells** (Gibco BRL, Cat. # 18265-017)
- **LB Amp-100 Broth** (Teknova, Cat. # L8100): 1.0% tryptone, 0.5% yeast extract, 1.0% NaCl with 100 µg/ml ampicillin
- **IPTG** (Teknova, Cat. #. I3425): isopropyl b-D-thiogalactopyranoside

3.2 Materials and Equipment

- **Microcentrifuge**
- **Sonicator**
- **Orbital shaker**
- **Hyperfilm™ ECL** (Amersham, Cat.# RPN1674K) or equivalent

OR

- **Chemiluminescence imaging system** (e.g., FluorChem™ from Alpha Innotech Corp.)

5. PREPARATION OF BACTERIAL EXPRESSION CONSTRUCT

Insert the PDZ ligand of interest into any commercially available histidine tag expression vector using standard molecular cloning techniques (13). Transform into DH5α competent cells with DNA ligation mix as described by the manufacturer's instructions, and screen for ligand-harboring bacteria using standard molecular techniques (13). After screening, transform the recombinant clone into the manufacturer's recommended strain for expression of fusion protein, e.g., BL21(DE3). We recommend checking that the transformed bacteria express ligand fusion protein by SDS-PAGE and/or by Western blot analysis.

6. PREPARATION OF BACTERIAL EXTRACTS WITH PDZ LIGAND

In this section, you will prepare bacterial extract containing your ligand of interest for hybridization with the array membrane (Section 7). For best results, we recommend that the protein concentration of your extract be at least 0.1-1 mg/ml.

6.1 Inoculate the transformed bacteria in 1 ml of LB/Amp (100 µg/ml) (Section 5).

6.2 Grow bacteria overnight at 37°C with shaking at 250 rpm.

6.3 Transfer 80 µl of the overnight culture to a tube containing 4 ml of LB/Amp (100 µg/ml).

6.4 Grow bacteria at 37°C until OD600 readings are approx. 0.5–0.8.

6.5 Add 100 µM–1mM IPTG.

6.6 Continue to grow for an additional 3–4 hr at 37°C.

6.7 Collect cells by centrifugation. Decant supernatant.

6.8 Resuspend the pellet in 750 µl of ice-cold 1X Resuspension Buffer.

6.9 Lyse cells using a sonicator.

6.10 Centrifuge at 14,000 rpm for 5 min at 4°C.

6.11 Transfer supernatant into a clean microcentrifuge tube.

6.12 Store on ice until further use. For longer storage, keep at –20°C.

7. INCUBATION

In this Section, you will incubate the bacterial extract containing your PDZ ligand of interest (prepared in Section 6) to the array membrane. Note that the array membranes have a notch at the top right-hand corner for orientation purposes.

Note: Be sure that the membrane is fully submerged in assay buffer at all times. Never let the membrane dry out.

7.1 Place each membrane into the provided four-well tray containing 5 ml of 1X Blocking Buffer. Make sure that the membrane is fully submerged in buffer.

7.2 Place the tray on a shaker and incubate for 1 hr at room temperature or overnight at 4°C.

7.3 Remove 1X Blocking Buffer, and briefly rinse membrane with 6 ml of 1X Wash Buffer.

7.4 Incubate the membrane with the diluted bacterial extract (or use the provided 20X Positive Control Extract diluted to 1X in 1X Resuspension Buffer) with gentle shaking for 1-2 hr at room temperature or overnight at 4°C.

7.5 After incubation, wash the membrane three times with 6 ml of 1X Wash Buffer for 10 min (each wash) at room temperature.

7.6 Incubate the membrane with 4 ml of 1X Anti-Hisitidine HRP Conjugate (diluted from 20X in 1X Wash Buffer) for 1-2 hr at room temperature.

7.7 Wash three times with 6 ml of 1X Wash Buffer for 10 min (each wash) at room temperature.

8. DETECTION

Important note: Do not let the membrane dry out during detection.

8.1 Prepare the detection solution immediately before use by mixing equal amounts of Detection Buffers A and B—e.g., 300 µl of Detection Buffer A and 300 µl of Detection Buffer B.

8.2 Using forceps to hold the cut corner, carefully remove each membrane from its tray. Drain the excess Wash Buffer from the membrane by touching the edge against tissue. Place protein- side up by orienting the notch to the top, right-hand corner on a clean plastic sheet.

8.3 Pipet the mixed Detection Buffers onto the membrane. Ensure that the buffer mixture is evenly distributed over the membrane without air bubbles.

8.4 Incubate for 5 min at room temperature.

8.5 Remove excess substrate by holding the membranes with forceps and touching the edge against tissue. Place the membrane between two plastic sheets and gently press on the top sheet to remove air bubbles.

8.6 Expose the membranes using either Hyperfilm™ ECL or a chemiluminescence imaging system, such as the FluorChem™ imager from Alpha Innotech Corp. In either case, we recommend that you try several different exposures of varying lengths of time (e.g., 30 sec–5 min).

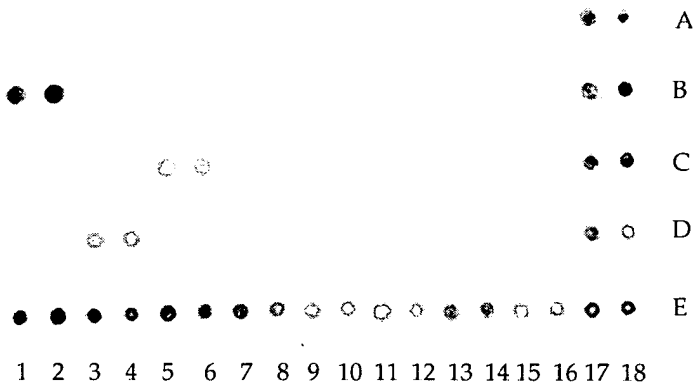
9. TROUBLESHOOTING GUIDE

Problem	Cause	Recommendation
Weak or no signal	Expressed ligand does not have a Histidine-tag.	Check construct by DNA sequencing. Ensure that the cloned insert does not contain an internal translational start site.
	Histidine-tag is partially hidden.	Protein binding may be hindered by a partially hidden Histidine-tag. Try using a high concentration (5-10X of the bacterial lysate) or longer binding time
High background	Concentration of bacterial lysate is too high.	Further dilute bacterial lysate (e.g. 30X).
	Antibody concentration is too high.	Further dilute the antibody.
	Nonspecific interactions with antibodies or other reagents used in the assay.	Check signal using a zero standard (i.e. PVDF membrane alone). High background is usually the result of insufficient blocking. Try longer incubation with the blocking buffer.
		Dilute the Detection Buffer.
Uneven background	Membrane dried out during incubation.	Keep the membrane fully submerged in solution during all incubation steps.
	Volume of blocking solution, bacterial lysate or antibody is too low.	Increase the volume to make sure that the membrane is fully submerged during incubation.
	Volume of detection buffer is too low.	Increase the volume to make sure that the membrane surface is fully covered.
	Air bubbles on membrane surface during detection.	Remove air bubbles from membrane surface.

10. REFERENCES

1. Cho, K.O. *et al.* (1992) The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. *Neuron* 9:929–942.
2. Woods, D.F. and Bryant, P.J. (1993) Zo-1, DlgA and PSD-5/SAP90: Homologous proteins in tight, septate and synaptic cell junctions. *Mech Dev* 44:889.
3. Kim, E. *et al.* (1995) Clustering of Shaker-type K+ channels by interaction with a family of membrane-associated guanylate kinases. *Nature* 378:85–88.
4. Kornau, H.C. *et al.* (1995) Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* 269:1737–1740.
5. Niethammer, M. *et al.* (1996) Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. *J Neurosci* 16:2157–2163.
6. Xu, X.Z. *et al.* (1998) Coordination of an array of signaling proteins through homo- and heteromeric interactions between PDZ domains and target proteins. *J Cell Biol* 142:545–555.
7. Hillier, B.J. *et al.* (1999) Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS-syntrophin complex. *Science* 284:812–815.
8. Christopherson, K.S. *et al.* (1999)PDS-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. *J Biol Chem* 274:27467–27473.
9. Fouassier, L. *et al.* (2000) Evidence for ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50) self-association through PDZ-PDZ interactions. *J Biol Chem* 275:25039–25045.
10. Bredt, D.S. (1998) Sorting out genes that regulate epithelial and neuronal polarity. *Cell* 94:691–694.
11. Fanning, A.S. and Anderson, J.M. (1998) PDZ domains and the formation of protein networks at the plasma membrane. *Curr Top Microbiol Immnol* 228:209–233.
12. Harris, B.Z. and Lim, W.A. (2001) Mechanism and role of PDZ domains in signaling complex assembly. *Journal of Cell Science* 114:3219–3231.
13. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning, a Laboratory Manual*, Second ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

APPENDIX A: Typical Results, Schematic Diagram & Domain List for PDZ Domain Array I



Typical results obtained with the TranSignal PDZ Domain Array I. *Class Kv1.4 PDZ ligand from bacterial extract specifically interacts with corresponding PDZ domains. Kv1.4 ligand was expressed as hisitine tag fusion protein. Bacterial extract was hybridized with the TranSignal PDZ Domain Array, and the image was acquired using FluorChem™ imager (from Alpha Innotech). Spots with stronger intensities indicate higher binding affinity of the ligand of interest to PDZ Domain(s).*

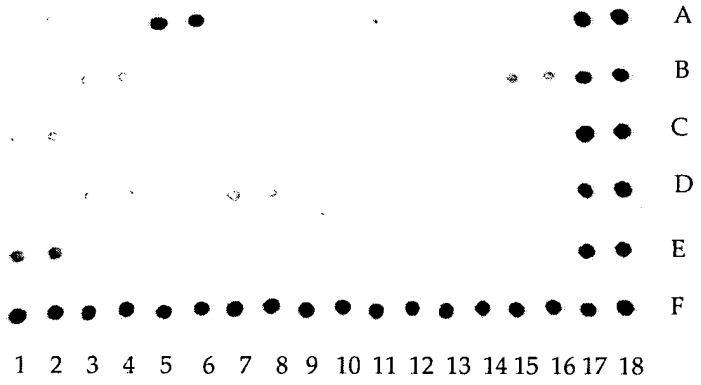
	1 | 2	3 | 4	5 | 6	7 | 8	9 | 10	11 | 12	13 | 14	15 | 16	17 | 18
A	Mint-2-D1	Mint-3-D1	Mint-3-D2	Mint-1-D1	Mint-1-D2	CSKP	Dlg-D1	Dlg1-D3	pos
B	Dlg2-D2	Dlg4-D3	DVL1	DVL3	DVLL	GIPC	HtrA2	LIMK2	pos
C	MPP2	NEB1	OMP25	hCLIM1	PTPH1	ZO-2-D1	hPTP1E-D1	hPTP1E-D5	pos
D	RGS12	RIL	ZO-1-D3	ZO-2-D3	GST				pos
E	pos	pos	pos	pos	pos	pos	pos	pos	pos

Schematic diagram of the TranSignal PDZ Domain Array I. *The proteins on the array are spotted in duplicate at 100 ng. Histidine-tagged ligand has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

PDZ domain list • TranSignal™ PDZ Domain Array I

POSITION	PDZ DOMAIN	FULL NAME
A1, 2	Mint-2-D1	X11L protein, PDZ domain #1
A3, 4	Mint-3-D1	X11L2 protein, PDZ domain #1
A5, 6	Mint-3-D2	X11L2 protein, PDZ domain #2
A7, 8	Mint-1-D1	X11 protein, PDZ domain #1
A9, 10	Mint-1-D2	X11 protein, PDZ domain #2
A11, 12	CSKP	Calcium/calmodulin-dependent serine protein kinase
A13, 14	Dlg-D1	Synapse-associated protein 97(SAP-97), PDZ domain #1
A15, 16	Dlg1-D3	Synapse-associated protein 97(SAP-97), PDZ domain #3
B1, 2	Dlg2-D2	Channel associated protein of synapse-110 (Chapsyn-110), PDZ domain #2
B3, 4	Dlg4-D3	Human postsynaptic density-95 (PSD-95), PDZ domain #3
B5, 6	DVL1	Dishevelled 1
B7, 8	DVL3	Dishevelled 3
B9, 10	DVLL	Dishevelled-1-like
B11, 12	GIPC	GAIP C-terminus interacting protein GIPC
B13, 14	HtrA2	High temperature requirement protein A2
B15, 16	LIMK2	LIM motif-containing protein kinase-2
C1, 2	MPP2	MAGUK p55 subfamily member 2
C3, 4	NEB1	Neurabin-I
C5, 6	OMP25	Mitochondrial outer membrane protein 25
C7, 8	hCLIM1	Human 36 kDa carboxyl terminal LIMdomain protein
C9, 10	PTPH1	Protein-tyrosine phosphatase H1
C11, 12	ZO-2-D1	Zonula occludens protein 2 , PDZ domain #1
C13, 14	hPTP1E-D1	Protein-tyrosine phosphatase 1E, PDZ domain #1
C15, 16	hPTP1E-D5	Protein-tyrosine phosphatase 1E, PDZ domain #5
D1, 2	RGS12	Regulator of G-protein signaling 12
D3, 4	RIL	Reversion-induced LIM protein
D5, 6	ZO-1-D3	Zonula occludens 1 protein, PDZ domain #3
D7, 8	ZO-2-D3	Zonula occludens protein 2, PDZ domain #3
D9, 10	GST	Glutathione-S-Transferase

APPENDIX B: Typical Results, Schematic Diagram & Domain List for PDZ Domain Array II



Typical results obtained with the TranSignal PDZ Domain Array II. *Class Kv1.4 PDZ ligand from bacterial extract specifically interacts with corresponding PDZ domains. Kv1.4 ligand was expressed as hisitine tag fusion protein. Bacterial extract was hybridized with the TranSignal PDZ Domain Array, and the image was acquired using FluorChem™ imager (from Alpha Innotech). Spots with stronger intensities indicate higher binding affinity of the ligand of interest to PDZ Domain(s).*

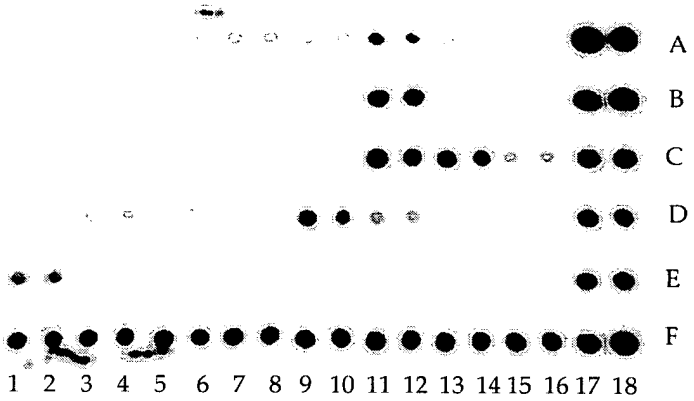
	1 | 2	3 | 4	5 | 6	7 | 8	9 | 10	11 | 12	13 | 14	15 | 16	17 | 18
A	KIAA0300-D6	KIAA0303	KIAA0316	KIAA0559	KIAA0613	KIAA1719-D7	KIAA1719-D4	KIAA1719-D3	pos
B	KIAA1526-D3	MAST205	KIAA1849	PALS1-D8	PALS1-D9	PALS1-D1	PALS1-D2	PALS1-D5	pos
C	PALS1-D3	MGC5395	FLJ23209-D1	FLJ23209-D2	KIAA1719-D6	FLJ00011	E3KARP-D1	E3KARP-21	pos
D	NHERF1-D1	Z01-D1	Z01-D2	SDB1-D1	SDB1-D2	IL16(2)-D3	LNX1-D3	NOS1	pos
E	PDZ-pos	GST							pos
F	pos	pos	pos	pos	pos	pos	pos	pos	pos

Schematic diagram of the TranSignal PDZ Domain Array II. *The proteins on the array are spotted in duplicate at 100 ng. Histidine-tagged ligand has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

PDZ domain list • TranSignal™ PDZ Domain Array II

POSITION	PDZ DOMAIN	ACCESSION	FULL NAME
A1, 2	KIAA0300-D6	BAA20760	KIAA0300 protein, Domain 6
A3, 4	KIAA0303	BAA20762	KIAA0303 protein
A5, 6	KIAA0316	BAA20774	KIAA0316 protein
A7, 8	KIAA0559	BAA25485	KIAA0559 protein
A9, 10	KIAA0613	BAA31588	KIAA0613 protein, Domain 7
A13, 14	KIAA1719-D4	BAB21810	KIAA1719 protein, Domain 4
A15, 16	KIAA1719-D3	BAB21810	KIAA1719 protein, Domain 3
B1, 2	KIAA1719-D6	BAB21810	KIAA1719 protein, Domain 6
B3, 4	KIAA1526-D3	BAA96050	KIAA1526 protein, Domain 3
B5, 6	MAST205	BAB40778	microtubule associated testis specific serine/ threonine protein kinase
B7,8	PALS1-D8	AAM28433	Pals1-associated tight junction protein, Domain 8
B9, 10	PALS1-D9	AAM28433	Pals1-associated tight junction protein, Domain 9
B11, 12	PALS1-D1	AAM28433	Pals1-associated tight junction protein, Domain 1
B13, 14	PALS1-D2	AAM28433	Pals1-associated tight junction protein, Domain 2
B15, 16	PALS1-D5	AAM28433	Pals1-associated tight junction protein, Domain 5
C1, 2	PALS1-D3	AAM28433	Pals1-associated tight junction protein, Domain 3
C3, 4	MGC5395	AAH12477	Similar to hypothetical protein MGC5395
C5, 6	FLJ23209-D1	NP_079171	hypothetical protein FLJ23209, Domain 1
C7, 8	FLJ23209-D2	NP_079171	Hypothetical protein FLJ23209, Domain 2
C9, 10	KIAA1719-D6	BAB21810	KIAA1719 protein, Domain 6
C11, 12	FLJ00011	Q9H7Q6	FLJ00011 protein (Fragment)
C13, 14	E3KARP-D1	NP_004776	solute carrier family 9 (sodium/hydrogen exchanger), 3 regulatory factor 2, Domain 1
C15, 16	E3KARP-D2	NP_004776	solute carrier family 9 (sodium/hydrogen exchanger), 3 regulatory factor 2, Domain 2
D1, 2	NHERF1-D1	NP_004243	solute carrier family 9 (sodium/hydrogen exchanger), Isoform 3 regulatory factor 1, Domain 1
D3, 4	Q07157	ZO1-D1	tight junction protein 1 (zona occludens), Domain 1
D5, 6	Q07157	ZO1-D2	tight junction protein 1 (zona occludens), Domain 2
D7, 8	NP_005616S	DB1-D1	syndecan binding protein (syntenin), melanoma differentiation associated protein-9, Pro-TGF-alpha cytoplasmic domain-interacting protein 18 (TACIP18), Domain 1
D9, 10	NP_005616S	DB1-D2	syndecan binding protein (syntenin), melanoma differentiation associated protein-9, Pro-TGF-alpha cytoplasmic domain-interacting protein 18 (TACIP18), Domain 2
D11, 12	NP_757366	IL16(2)-D3	interleukin 16 isoform 2; lymphocyte chemoattractant factor, Domain 3
D13, 14	Q8TBB1	LNX1-D3	Numb-binding protein 1; Ligand of Numb-protein, Domain 3
D15, 16	NOS1	NP_000611	nitric oxide synthase 1 (neuronal), domain 5
E1, 2	PDZ-pos		PDZ Domain positive control for Kv1.4 ligand
E3, 4	GST		Glutathione-S-Transferase

APPENDIX C: Typical Results, Schematic Diagram & Domain List for PDZ Domain Array III



Typical results obtained with the TranSignal PDZ Domain Array III. *Class Kv1.4 PDZ ligand from bacterial extract specifically interacts with corresponding PDZ domains. Kv1.4 ligand was expressed as hisitine tag fusion protein. Bacterial extract was hybridized with the TranSignal PDZ Domain Array, and the image was acquired using FluorChem™ imager (from Alpha Innotech). Spots with stronger intensities indicate higher binding affinity of the ligand of interest to PDZ Domain(s).*

	1 | 2	3 | 4	5 | 6	7 | 8	9 | 10	11 | 12	13 | 14	15 | 16	17 | 18
A	MAGI3-D2	MAGI3-D4	MAGI3-D5	MAGI3-D6	BAI1-D2	BAI1-D3	BAI1-D4	BAI1-D1	pos
B	BAI1-D6	AIP1-D2	AIP1-D3	AIP1-D4	AIP1-D5	AIP1-D6	hPTP1E-D2	hPTP1E-D3	pos
C	hPTP1E-D4	PTPN4	GRIP1-D4	GRIP1-D3	GRIP1-D2	SCRIB1-D1	SCRIB1-D2	SCRIB1-D4	pos
D	PARD3-D2	PARD3-D3	HARM-D3	MLL4	TIP1	SDB2-D2			pos
E	PDZ-pos	GST							pos
F	pos	pos	pos	pos	pos	pos	pos	pos	pos

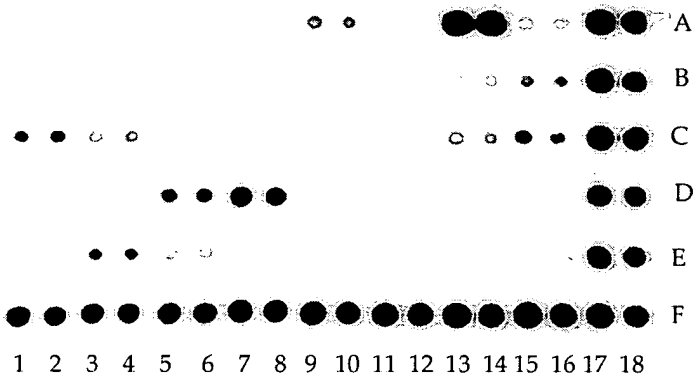
Schematic diagram of the TranSignal PDZ Domain Array III. *The proteins on the array are spotted in duplicate at 100 ng. Histidine-tagged ligand has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

PDZ domain list • TranSignal™ PDZ Domain Array III

POSITION	PDZ DOMAIN	ACCESSION	FULL NAME
A1, 2	MAGI3-D2	NP_690864	membrane-associated guanylate kinase-related 3, Domain 2
A3, 4	MAGI3-D4	NP_690864	membrane-associated guanylate kinase-related 3, Domain 4
A5, 6	MAGI3-D5	NP_690864	membrane-associated guanylate kinase-related 3, Domain 5
A7, 8	MAGI3-D6	NP_690864	membrane-associated guanylate kinase-related 3, Domain 6
A9, 10	BAI1-D2	NP_004733	brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 2
A11, 12	BAI1-D3	NP_004733	brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 3
A13, 14	BAI1-D4	NP_004733	brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 4
A15, 16	BAI1-D1	NP_004733	brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 1
B1, 2	BAI1-D6	NP_004733	brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 6
B3, 4	AIP1-D2	NP_036433	atrophin-1 interacting protein 1, Domain 2
B5, 6	AIP1-D3	NP_036433	atrophin-1 interacting protein 1, Domain 3
B7, 8	AIP1-D4	NP_036433	atrophin-1 interacting protein 1, Domain 4
B9, 10	AIP1-D5	NP_036433	atrophin-1 interacting protein 1, Domain 5
B11, 12	AIP1-D6	NP_036433	atrophin-1 interacting protein 1, Domain 6
B13, 14	hPTP1E-D2	NP_542416	protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1; protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 2
B15, 16	hPTP1E-D3	NP_542416	protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1; protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 3
C1, 2	hPTP1E-D4	NP_542416	protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1; protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 4
C3, 4	PTPN4	NP_002821	protein tyrosine phosphatase, non-receptor type 4; megakaryocyte phosphatase; PTPase-MEG1
C5, 6	GRIP1-D4	CAB39895	GRIP1 protein, Domain 4
C7, 8	GRIP1-D3	CAB39895	GRIP1 protein, Domain 3
C9, 10	GRIP1-D2	CAB39895	GRIP1 protein, Domain 2
C11, 12	SCRIB1-D1	NP_056171	Scribble, Domain 1
C13, 14	SCRIB1-D2	NP_056171	Scribble, Domain 2
C15, 16	SCRIB1-D4	NP_056171	Scribble, Domain 4

POSITION	PDZ DOMAIN	ACCESSION	FULL NAME
D1, 2	PARD3-D2	NP_062565	partitioning-defective protein 3 homolog; atypical PKC isotype-specific interacting protein, Domain 2
D3, 4	PARD3-D3	NP_062565	partitioning-defective protein 3 homolog; atypical PKC isotype-specific interacting protein, Domain 3
D5, 6	HARM-D3	NP_005700	harmonin; PDZ-73 protein; antigen NY-CO-38, Domain 3
D7, 8	MLL4	NP_005927	myeloid/lymphoid or mixed-lineage leukemia, translocated to, 4
D9, 10	TIP1	NP_055419	Tax interaction protein 1
D11, 12	SDB2-D2	NP_056500	syntenin-2beta; syntenin-2; similar to syndecan binding protein, Domain 2
E1, 2	PDZ-pos		PDZ Domain positive control for Kv1.4 ligand
E3, 4	GST only		Glutathione-S-Transferase

APPENDIX D: Typical Results, Schematic Diagram & Domain List for PDZ Domain Array IV



Typical results obtained with the TranSignal PDZ Domain Array IV. *Class Kv1.4 PDZ ligand from bacterial extract specifically interacts with corresponding PDZ domains. Kv1.4 ligand was expressed as hisitine tag fusion protein. Bacterial extract was hybridized with the TranSignal PDZ Domain Array, and the image was acquired using FluorChem™ imager (from Alpha Innotech). Spots with stronger intensities indicate higher binding affinity of the ligand of interest to PDZ Domain(s).*

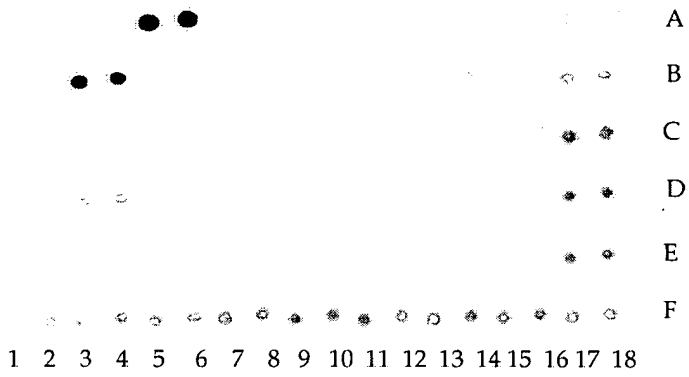
	1 | 2	3 | 4	5 | 6	7 | 8	9 | 10	11 | 12	13 | 14	15 | 16	17 | 18
A	MUPP1-D6	MUPP1-D12	MUPP1-D2	MUPP1-D3	MUPP1-D13	MUPP1-D1	DLG3-D2	DLG3-D3	pos
B	DLG5-D1	DLG5-D4	DLG5-D3	DLG5-D2	DLG2-D3	PAR6B	LIK1	LOMP	pos
C	RIL	A2LIM	TIAM1	LIN7C	LIN7B	LIN7A	GEF11	GEF12	pos
D	PDZK1-D1	PDZK1-D2	SNB1	SNA1	SHK1	MPP6	PIST	GEF2	pos
E	RIM2	PDZ-pos	GST						pos
F	pos	pos	pos	pos	pos	pos	pos	pos	pos

Schematic diagram of the TranSignal PDZ Domain Array IV. *The proteins on the array are spotted in duplicate at 100 ng. Histidine-tagged ligand has been spotted along the bottom (row F) and in duplicate along the right side (column 17, 18) of the membrane. These spots are intended for alignment. Note that the notch is at the top right-hand corner.*

PDZ domain list • TranSignal™ PDZ Domain Array IV

POSITION	PDZ DOMAIN	ACCESSION	FULL NAME
A1, 2	MUPP1-D6	NP_003820	multiple PDZ domain protein, Domain 6
A3, 4	MUPP1-D12	NP_003820	multiple PDZ domain protein, Domain 12
A5, 6	MUPP1-D2	NP_003820	multiple PDZ domain protein, Domain 2
A7, 8	MUPP1-D3	NP_003820	multiple PDZ domain protein, Domain 3
A9, 10	MUPP1-D13	NP_003820	multiple PDZ domain protein, Domain 13
A11, 12	MUPP1-D1	NP_003820	multiple PDZ domain protein, Domain 1
A13, 14	DLG3-D2	NP_066943	synapse-associated protein 102; neuroendocrine-dlg; discs large homolog 3, Domain 2
A15, 16	DLG3-D3	NP_066943	synapse-associated protein 102; neuroendocrine-dlg; discs large homolog 3, Domain 3
B1, 2	DLG5-D1	NP_004738	discs, large homolog 5, Domain 1
B3, 4	DLG5-D4	NP_004738	discs, large homolog 5, Domain 4
B5, 6	DLG5-D3	NP_004738	discs, large homolog 5, Domain 3
B7, 8	DLG5-D2	NP_004738	discs, large homolog 5, Domain 2
B9, 10	DLG2-D3	NP_001355	Channel associated protein of synapse-110 (Chapsyn-110), Domain 3
B11, 12	PAR6B	BAB40756	Partitioning defective-6 homolog beta Domain 1
B13, 14	LIK1	NP_002305	LIM domain kinase 1 isoform 1; LIM motif-containing protein kinase
B15, 16	LOMP	NP_005349	LIM domain only 7 isoform a; KIAA0858 protein
C1, 2	RIL	NP_003678	LIM protein RIL (Reversion-induced LIM protein)
C3, 4	A2LIM	NP_055291	alpha-actinin-2-associated LIM protein; enigma homolog
C5, 6	TIAM1	NP_003244	T-cell lymphoma invasion and metastasis 1
C7, 8	LIN7C	NP_060832	Lin-7 homolog C
C9, 10	LIN7B	NP_071448	Lin-7 homolog B
C11, 12	LIN7A	NP_004655	Lin-7 homolog A
C13, 14	GEF11	NP_055599	Rho guanine exchange factor (GEF) 11; glutamate transporter EAAT4-associated protein 48; KIAA0380 protein
C15, 16	GEF12	NP_056128	Rho guanine exchange factor (GEF) 12; leukemia-associated GEF; similar to mouse Lsc oncogene
D1, 2	PDZK1-D1	NP_002605	PDZ domain containing 1, Domain 1
D3, 4	PDZK1-D2	NP_002605	PDZ domain containing 1, Domain 2
D5, 6	SNB1	NP_066301	Beta-1-syntrophin; tax interaction protein 43; dystrophin-associated protein A1, 59kD, basic component 1
D7, 8	SNA1	NP_003089	acidic alpha 1 syntrophin; dystrophin-associated protein A1, 59kD, acidic component; pro-TGF-alpha cytoplasmic domain-interacting protein 1
D9, 10	SHK1	NP_057232	somatostatin receptor-interacting protein; SH3 and multiple ankyrin repeat domains 1
D11, 12	MPP6	NP_057531	membrane protein, palmitoylated 6; protein associated with Lin7 2; VELI-associated MAGUK 1; MAGUK protein p55T
D13, 14	PIST	NP_065132	Golgi associated and coiled-coil motif containing protein; CFTR-associated PDZ/coiled-coil domain binding partner for the rho-family GTPase TC10; fused in glioblastoma; Golgi associated PDZ and coiled-coil motif containing protein
D15, 16	GEF2	NP_057424	Rap guanine nucleotide exchange factor; PDZ domain-containing guanine nucleotide exchange factor I
E1, 2	RIM2	NP_055492	regulating synaptic membrane exocytosis 2; RAB3 interacting protein 3; KIAA0751 protein
E3, 4	PDZ-pos		PDZ Domain positive control for Kv1.4 ligand
E3, 4	GST		Glutathione-S-Transferase

APPENDIX E: Biotin-Conjugated Peptide with PDZ Domain Arrays



Binding of a biotin-conjugated PDZ ligand peptide on PDZ Domain Array II. *Membrane was incubated with Blocking Buffer for 1 h, rinsed once with 1X Wash Buffer and probed with Peptide Probe Mix for 1 h (Peptide Probe Mix: mix 15 ul of 0.1 mM biotin-conjugated peptide with 1 mg/ml Strepavidin-HRP; incubate with gentle rocking for 30 min at 4°C; and add to 12 ml of 1X Blocking Buffer). Membrane was washed three times with 1X Wash Buffer after incubation with the peptide. Chemiluminescent signal was detected as described in Section 8 and the image was aquired using FluorChem™ imager (from Alpha Innotech).*

NOTES: